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Christine Bellanné-Chantelot, Cécile Saint-Martin, Maria-Joao Ribeiro, Christelle Vaury, Virginie Verkarre, Jean-Baptiste Arnoux, Vassili Valayannopoulos, Sandrine Gobrecht, Christine Sempoux, Jacques Rahier, et al.

Title

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Christine Bellanné-Chantelot,[1*] Cécile Saint-Martin,[1] Maria-Joao Ribeiro,[2] Christelle Vaury,[1] Virginie Verkarre,[3] Jean-Baptiste Arnoux,[4] Vassili Valayannopoulos,[4] Sandrine Gobrecht,[1] Christine Sempoux,[5] Jacques Rahier,[5] Jean-Christophe Fournet,[3] Francis Jaubert,[3] Yves Aigrain[6], Claire Nihoul-Fékété,[6] and Pascale de Lonlay[4]

***Corresponding author**

Dr Christine Bellanné-Chantelot

Centre de Génétique Moléculaire et Chromosomique

Groupe Hospitalier Pitié Salpêtrière, 47-83 bd de l'Hôpital, 75651 PARIS Cedex 13, FRANCE

Phone: +33 1 42 17 76 52

Fax: +33 1 42 17 76 18

E-mail: christine.bellanne-chantelot@psl.aphp.fr

Authors' affiliations

[1] Department of Genetics, AP-HP Groupe Hospitalier Pitié-Salpétrière, Université Pierre et Marie Curie, Paris, France

[2] Service Hospitalier Frédéric Joliot, I2BM, CEA, Orsay, France

[3] Department of Pathology, AP-HP Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, France

[4] Reference Center for Inherited Metabolic Diseases, AP-HP Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, France

[5] Department of Pathology, Faculty of Medicine, University of Louvain, Brussels, Belgium

[6] Department of Paediatric Surgery, AP-HP Hôpital Necker-Enfants Malades, Université Paris Descartes, Paris, France

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ABSTRACT

Background: Congenital hyperinsulinism (CHI) is characterized by an over secretion of insulin by the pancreatic β-cells. This condition is mostly caused by mutations in *ABCC8* or *KCNJ11* genes encoding the SUR1 and KIR6.2 subunits of the ATP-sensitive potassium (K_{ATP}) channel. CHI patients are classified according to their responsiveness to diazoxide and to their histopathological diagnosis (either focal, diffuse or atypical forms). Here, we raise the benefits/limits of the genetic diagnosis in the clinical management of CHI patients.

Methods: *ABCC8*/*KCNJ11* mutational spectrum was established in 109 diazoxide-unresponsive CHI patients for whom an appropriate clinical management is essential to prevent brain damage. Relationships between genotype and radiopathological diagnosis were analysed.

Results: *ABCC8* or *KCNJ11* defects were found in 82% of the CHI cases. All patients with a focal form were associated with a single K_{ATP} channel molecular event. In contrast, patients with diffuse forms were genetically more heterogeneous: 47% were associated with recessively inherited mutations, 34% carried a single heterozygous mutation and 19% had no mutation. There appeared to be a predominance of paternally inherited mutations in patients diagnosed with a diffuse form and carrying a sole K_{ATP} channel mutation.

Conclusions: The identification of recessively inherited mutations related to severe and diffuse forms of CHI provides an informative genetic diagnosis and allows prenatal diagnosis. In contrast, in patients carrying a single K_{ATP} channel mutation, genetic analysis should be confronted with the PET imaging to categorize patients as focal or diffuse forms in order to get the appropriate therapeutic management.

Congenital hyperinsulinism (CHI; MIM #256450) characterized by an inappropriate over secretion of insulin from pancreatic β-cells is the most frequent cause of persistent hypoglycaemia with an estimated incidence of 1 / 50 000 births in most populations.(1, 2) The clinical challenge concerns patients who are unresponsive to the medical treatment, as severe and persistent hypoglycaemias lead to irreversible brain damage.

CHI is related to different genetic aetiologies. In rare cases, CHI results from anomalies of either the glucokinase (*GCK*), the glutamate dehydrogenase (*GLUD1*), short-chain L-3-hydroxyacyl-CoA dehydrogenase (*HADH*) genes (3 for review) or the hepatocyte nuclear factor 4A (*HNF4A*), the latter being associated with transient forms of CHI (4), and more recently the monocarboxylate transporter 1 (*SLC16A1)*, which causes exercise-induced HI.(5) However, CHI is mostly associated with molecular defects of the β-cell ATP-sensitive potassium (K_{ATP}) channel genes, *KCNJ11* and *ABCC8*, respectively encoding the channel subunits Kir6.2 and SUR1.(2, 6-9)

The management of patients depends on the responsiveness to medical therapy. The treatment of CHI is primarily based on the use of diazoxide, a K_{ATP} channel agonist. Since *ABCC8* and *KCNJ11* defects can impair the channel response to its agonist,(10) another alternative is to use octreotide and frequent or continuous feeding.(11) Pancreatic surgery should be restricted to medically unresponsive CHI. It is therefore essential since their medical care will be different to classify patients correctly according to the two main histopathological forms of CHI.(12-14) The focal form consists of abnormal pancreatic β-cells restricted to a limited pancreatic area and can be cured by the targeted removal of the pathological tissue. In contrast, the diffuse form affects all the β-cells and thus requires near total pancreatectomy when patients are unresponsive to an intensive medical treatment. Besides these two well-defined forms of CHI, some histopathological forms are not well-

characterized, neither being typically focal nor typically diffuse, some of them presenting a mosaicism of their pancreatic β-cells resulting in an atypical form of CHI.(15-17)

Focal and diffuse forms of CHI related to a K_{ATP} channel defect involve distinct molecular mechanisms. Focal forms are sporadic defects resulting from two events: a paternally inherited *KCNJ11* or *ABCC8* mutation and the loss of the corresponding maternal allele in some pancreatic β-cells leading to a focal lesion.(18, 19) Diffuse forms are classically associated with an autosomal recessive mode of inheritance.(6, 8, 20) Both focal CHI and recessively inherited CHI related to a K_{ATP} channel defect are usually resistant to diazoxide.(8) By contrast, the rare diffuse forms described with a dominant inheritance are sensitive to diazoxide.(21, 22) Altogether, *ABCC8* and *KCNJ11* mutations would account for about 50% of CHI patients.(2, 8) However, the proportion of K_{ATP} channel mutations identified in CHI remains inaccurately defined since large studies have not been performed by sequence analysis, and most mutations were reported through case reports.(9) Furthermore, CHI cases related to *ABCC8/KCNJ11* mutations were mostly described independently of their responsiveness to diazoxide.

Here, we describe the *ABCC8* and *KCNJ11* mutational spectrum in a large series of diazoxide-unresponsive CHI patients. We discuss the benefits/limits of the *ABCC8/KCNJ11* genetic testing according to the radiological and histological diagnosis, and the clinical situations that warrant a molecular analysis to improve the clinical management of diazoxide-unresponsive CHI patients.

METHODS

Patients

From 1999 to 2009, we studied 109 diazoxide-unresponsive unrelated CHI patients (57 boys, 52 girls) who were referred for clinical and/or radiological investigations or surgery to Necker-Enfants Malades Hospital (Paris, France). Most of them (92/109, 84.4%) were diagnosed during the neonatal period, whereas 16 patients were diagnosed between 1 and 6 months of life, and 1 girl at 22 months. The series includes 87 Euro-Caucasians (80%), 13 North-Africans, 3 Turkish, 3 Asians, 1 African and 2 from the Indian Ocean region.

Clinical diagnosis of CHI was based on the observation of recurrent non ketotic hypoglycemias (blood glucose concentration < 3 mmol/l) associated with concomitant inappropriate plasma insulin concentration.(8) Non-responsiveness to diazoxide was defined as two confirmed blood glucose measurements lower than 3 mmol/l in a 24-hours period after at least five consecutive days of diazoxide therapy at 15 mg/kg/day (neonates) or 10 mg/kg/day (infants) divided in 3 oral doses.(8) Patients with syndromic CHI (e.g. hyperinsulinism-hyperammonemia (HI/HA) syndrome or CHI associated with overgrowth syndromes) were excluded, as each syndrome warrants its specific molecular testing and because they are diazoxide-sensitive. For all studied patients, we obtained the written informed consent in accordance with the French ethical guidelines.

Radiological and histopathological diagnosis

Radiological investigations were based on transhepatic catheterization of the portal and pancreatic veins with pancreatic veins sampling (PVS) for patients explored before 2005 and positons emission tomography (PET) performed with [^{18}F] fluoro-L-DOPA since 2005.(14, 23-26) In cases treated by surgery, the histological diagnosis (focal, diffuse or atypical form)

was assessed by analysis of intraoperative frozen samples, formalin fixed and paraffin embedded sections, as described.(12, 15, 27).

All the pancreatic tissue samples were analysed by two pediatric pathologists, at the time of surgery and before inclusion in this study. We subdivided CHI patients into three radiopathological groups. The first one consisted of 37 patients with a focal form of CHI, 35 of them underwent surgery. The second group included 64 patients with a diffuse form of CHI. In this group, patients were subclassified in “diffuse CHI” (27 operated patients, with pathological confirmation) and “suspected diffuse CHI” (37 patients with radiological diagnosis and no histological confirmation). The third group consisted of 8 operated patients with an atypical histopathological form as previously described.(17, 28)

Median values of clinical characteristics were used for analysis. Categorical data were compared with Fisher exact test. A p value of less than 0.05 was considered of statistical significance.

Genetic analysis

The search for germline events was performed on genomic DNA extracted from peripheral lymphocytes. The study followed five steps:

(i) Sequencing of the coding sequence and exon/intron boundaries of *KCNJ11* and *ABCC8* genes and the *ABCC8* promoter region in probands. In four patients, sequence analysis was also performed on pancreatic DNA extracted from frozen sections to search for somatic events.

(ii) Variant validation. First, we performed bioinformatic analysis using Polyphen, Sift and GVGD for missense variants; SpliceSiteFinder, NNSPLICE, GeneSplicer and MaxEntScan for splice site variants. Variants were considered as mutations when these algorithms

predicted them to be deleterious. Second, the variants were excluded from 400 geographically-matched control chromosomes.

(iii) Cosegregation analysis in the parents. In case of a suspected *de novo* event, we genotyped 14 microsatellite markers (Individual Panel 15 for Linkage Mapping Set v2.5, Applied Biosystems) including 6 markers on chromosome 11 in both patient and parents.

(iv) In all probands except those with compound heterozygous mutations, search for *ABCC8* genomic rearrangements by multiplex ligation-dependent probe assay (MLPA)

(v) *GCK* screening in patients with no *ABCC8* and *KCNJ11* mutation.

Sequence analysis

KCNJ11 and *ABCC8* sequencing. The sequence analysis included first the amplification by polymerase chain reaction (PCR) of the single exon of *KCNJ11*, the promoter region of *ABCC8* and its 39 exons. The purified PCR products were sequenced in both directions using the ABI PRISM BigDye Terminator chemistry (Applied Biosystems). Sequencing reactions were run on an ABI3100 Genetic Analyzer and analyzed with the Seqscape software Version 2.2 (Applied Biosystems). The mutation nomenclature is based on the reference sequences *KCNJ11* NM_000525.3 and *ABCC8* NM_000352.3 corresponding to the L72808 isoform (1,582 amino acids) which incorporates the extra serine residue in exon 17.(9)

GCK sequencing. The sequence analysis of the coding exons of the pancreatic isoform of *GCK* (NM_000162.3) was performed for all patients with normal *KCNJ11* and *ABCC8* investigation.

All primer sequences and amplification conditions are available on request.

MLPA analysis

We used the SALSA MLPA KIT P117 *ABCC8* (MRC Holland, The Netherlands) for the search for genomic rearrangements. The procedure was carried out according to manufacturer's instructions. Ligation products were separated on an ABI3730 Genetic

Analyzer. The analysis was performed using GeneMapper, Version 4.0 (Applied Biosystems). Single-exon deletions were checked by real-time quantitative PCR based on SYBR-Green I fluorescence.

Microsatellite marker analysis

A panel of 11 microsatellite markers from the chromosome 11-region around *ABCC8* and *KCNJ11* (D11S2071, D11S1363, D11S922, D11S2344, D11S2347, D11S1901, D11S419, D11S1397, D11S921, D11S902 and D11S1888) were analyzed on DNA extracted from leucocytes and pancreatic frozen sections. The analysis was performed using GeneMapper, Version 4.0.

RESULTS

We identified a K_{ATP} channel defect in 89 out of 109 probands (81.6%), most of them involving the *ABCC8* gene (79/89, 88.8 %). Two mutations were found in 28% (30/109) of the probands, which is therefore consistent with a recessive mode of inheritance (table 1). Among them, 11/30 had a homozygous mutation, 5/11 belonging to a consanguineous family and 19/30 patients were compound heterozygotes. In 59 probands (59/109, 54%), a single heterozygous mutation was identified (table 1). The analysis of both parents for 41/59 showed 35/41 (85%) paternally inherited mutations and 1/41 maternally inherited mutation. *De novo* *ABCC8* mutations occurred in 5/41 probands (12.2%). Lastly, no evidence for K_{ATP} channel defect was found in 20/109 (18%) probands. A *de novo GCK* mutation was identified in one of these.

Characteristics of *ABCC8* and *KCNJ11* molecular defects

Eighty-nine probands were identified with K_{ATP} channel defects. A total of 118 mutations were found, including 106 *ABCC8* mutations (90%) and 12 *KCNJ11* mutations (10%) (table 2). Ninety-four out of 118 were different mutations, 41 were previously reported. Out of the 53 (56%) new mutations, 47 (*ABCC8*, n = 40 and *KCNJ11*, n= 7, table 2) were only observed once in our series.

Eighty-one different *ABCC8* point mutations were identified (table 2). Regarding their type, 39/81 (48%) were missense mutations whereas 42/81 (49%) were either truncating mutations predicted to generate premature stop codons (15 nonsense mutations, 8 out-of-frame mutations and 14 splice site defects) or in-frame small deletions/duplications (5/42). Most *ABCC8* missense mutations (74%) were located in the nucleotide-binding domains (NBD1, amino acids 679-929 and NBD2, amino acids 1344-1578), particularly in the second domain whereas only 38% of truncating mutations affected those domains (14/37, Table 2).

Eleven different *KCNJ11* mutations, 10 missense and 1 frameshift mutations were identified in 12 probands (table 2). One mutation, p.Gln128Arg, was located in the pore-forming helix; one was in an extra-cellular loop and affects a residue strongly involved in the interaction of K^+ along the pore (p.Arg136Cys)(29); other mutations were located in the cytoplasmic domains.

Patients with normal *ABCC8* and *KCNJ11* sequencing and those carrying a single *ABCC8* heterozygous mutation were investigated in search for *ABCC8* deletions. We detected by MLPA technique and confirmed by fluorescent quantitative PCR single-exon deletions of *ABCC8* in two compound heterozygous patients: a deletion of exon 8 (p.Thr393_Gln444del52) and a deletion of exon 22 (p.Asp854_Trp899del46) (table 2 and Supplemental material). In order to exclude *KCNJ11* deletions in negative patients, four recurrent polymorphisms located in the *KCNJ11* coding sequence were analysed to rule out a possible hemizygosity. Ten patients were homozygous for the *KCNJ11* gene. Gene deletion could be excluded for all of them using the fluorescent quantitative multiplex PCR method.

Correlation between the *ABCC8*/*KCNJ11* genotype and the radiopathological diagnosis

Mutations were dispersed throughout the *ABCC8* and *KCNJ11* genes in focal forms as well as in diffuse or suspected diffuse forms. Most mutations were observed only once, thus very few mutations were identified in both focal and diffuse (c.428G>A, c.536A>G, c.1792C>T, c.3133_3152del20 and c.3644G>A) or suspected diffuse (c.1630+1G>T) forms (table 2).

The 37/109 patients diagnosed with a focal form were all associated with a unique heterozygous mutation affecting the K_{ATP} channel, 32 detected in *ABCC8* and 5 in *KCNJ11* (tables 1 and 2). Mutations were paternally inherited (24 out of 26 tested parents) or arose *de novo* (2/26). All were distinct, 46% of them (17/37) were missense mutations whereas 51% were truncating (19/37) and one led to a deletion of two amino acids (table 2).

Among the 64/109 cases diagnosed with a diffuse form (confirmed by histology, 27/64 patients) or suspected to have a diffuse CHI (on PET or PVS examination, 37 patients), 47% (30/64) were either homozygotes (11/30) or compound heterozygotes (19/30), 34% (22/64) carried a single heterozygous mutation and 19% (12/64) had none (table 1). No significant difference was observed in the number (2, 1 and 0) of K_{ATP} mutations identified between patients with a diagnosis of diffuse form proved by histology and those with a diagnosis of diffuse form based on radiological investigation only (table 1). In patients with a recessive form of CHI, 55% of K_{ATP} mutations were missense mutations whereas 45% were truncating. In contrast, truncating mutations were less frequently observed in patients carrying a single K_{ATP} heterozygous mutation (23% vs. 45%).

Among the 22 patients with a single heterozygous K_{ATP} channel mutation, 10 had a diagnosis of diffuse CHI confirmed by histology. For 5 of them, the segregation analysis of both parents showed that the mutation had arisen *de novo* in 2 and was paternally inherited in 3 (table 1). To exclude a focal form, we analyzed 11 microsatellite markers in the pancreatic tissue of four patients compared to 2 patients with focal forms and 1 patient with a diffuse form due to 2 mutations. Our results showed a loss of the maternal allele in the two focal forms, as expected, while in the diffuse forms no difference was found between patients with one or two mutations (supplemental material). Futhermore, pathological comparisons between pancreatic specimens from diffuse cases with two mutations and those with one mutation showed the same features characterized by enlarged pancreatic β-cell nuclei throughout the entire pancreas. We also excluded a somatic coding mutation by sequencing *ABCC8* and *KCNJ11* of pancreatic DNA of these four patients.

In the 12 patients with diffuse form of CHI suspected by PET imaging and carrying a heterozygous *ABCC8* mutation, segregation analysis of both parents for 10 cases showed that the mutation had arisen *de novo* in 1 case, was maternally inherited in 1 and paternally

inherited in 8 (table 1). In the maternally inherited case, several episodes of hypoglycemia-related seizures in infancy were reported in the mother, although she was asymptomatic at the age of 28. There is strong evidence that the identified mutation Gly716Asp is pathogenic: (i) it is located in the first nucleotide binding domain. (ii) the same residue was reported to be altered (Gly716Val) in a homozygous recessive form of CHI resistant to diazoxide (30) and (iii) the functional study of Gly716Val showed a reduced surface expression of the mutant channel.(31)

Among the 8 paternally inherited cases, no clinical symptom was reported in the fathers at the time of the child's diagnosis. Two families displayed the recurrence of severe CHI. For one of them, DNA was available from the affected sibling; the analysis of six microsatellite markers located in 11p15.1 showed that they did not share the same maternal haplotype and thus excluded a recessive inheritance (data not shown).

Last, no *ABCC8/KCNJ11* mutation was found in 3 patients with a diagnosis of diffuse form and in 9 with diffuse form suspected by PET (table 1). One of the operated patients was subsequently found to carry a *de novo GCK* mutation, c.191C>T; p.Ser64Phe.

Finally, no germline *ABCC8/KCNJ11* and *GCK* mutations were identified in the 8 patients with a histological diagnosis of atypical CHI. Paternal isodisomy at the *ABCC8* locus was excluded at the pancreatic level for 3 of them. Since one patient had a monozygotic healthy twin, we also excluded the presence of a somatic mutation.

DISCUSSION

We have shown that mutations in the *ABCC8* and *KCNJ11* genes respectively encoding the SUR1 and KIR6.2 subunits of the K_{ATP} channel are major causes of CHI refractory to diazoxide, as they account for 82% of all cases in a large series of 109 patients. This estimate is higher than those previously reported in several series in which a molecular aetiology was found in about 50% to 65% of cases.(2, 8, 32-34) This discrepancy is probably due to differences in the clinical phenotype of patients, particularly their response to medical treatment, and in the sensitivity of the screening method used for the detection of mutations.

Our study shows that *ABCC8* gene defects are the most important cause of diazoxide-unresponsive CHI (89% of mutated patients). The mutational spectrum underlines the allelic diversity of *ABCC8* and *KCNJ11* mutations since 80% of identified molecular events were distinct in this series and half of them were not reported in the recent update of K_{ATP} channel mutations (9) or in subsequent publications. A genomic rearrangement was identified in only two recessive forms of CHI, each one deleting a single exon, demonstrating the rarity of large deletions in CHI.

We classified CHI patients into three groups according to *ABCC8*/*KCNJ11* mutation status (2 recessively inherited mutations, 1 single mutation and no mutation) and investigated the correlation between genotypes and histopathological or radiological diagnosis.

In 28% of patients (30/109), we found recessively inherited mutations, either homozygous or compound heterozygous, all associated with a diagnosis of diffuse form of CHI. Nearly two third of these patients were refractory to all medical treatment and consequently, required near-total pancreatectomy. These patients characterized by a severe clinical presentation were also frequently large for gestational age. Half of them had a birth weight above 4,000 g.

In 54% of patients (59/109), we detected a single mutation in the coding sequence and boundary regions of *ABCC8* or *KCNJ11*. We studied the allelic segregation in 41 families and

found that 12% arose *de novo*, one was maternally inherited and 85% of mutations were paternally inherited. The 37 patients diagnosed with a focal form were all included in this group. This result emphasized the absence of genetic heterogeneity of focal forms of CHI which are exclusively related to functional abnormalities of the K_{ATP} channel. This corroborates our previous results (8, 19) and a recent study by Suchi *et al*. who found a K_{ATP} channel gene mutation in 86% of patients with a focal form.(35)

The 22 remaining patients had a diagnosis of either diffuse form (n=10) proved on histological examination or suspected diffuse form (n=12) based on PET-scan analysis. To explain the identification of a unique KATP mutation, we considered different hypotheses: (i) misdiagnosed focal forms, (ii) dominant forms of CHI, (iii) unidentified second recessive mutation and (iv) post-zygotic event. Firstly, we excluded the specific features of the focal forms at the histological and molecular levels. In the ten patients who had surgery, a diffuse lesion was confirmed by pathological examination of the pancreatic tissue showing a pattern dramatically different from a focal lesion. At the molecular level, the analysis of microsatellite markers excluded any loss of the maternal allele. Additionally, we and others have demonstrated the accuracy of the PET imaging on both focal and diffuse forms of CHI.(25, 26, 36-37). Altogether, our results did not support a bias related to the type of investigation since we observed a similar proportion of single mutations in diffuse forms in both operated (10/27) and non operated patients (12/37). Secondly, segregation analysis of 5 diffuse forms and 10 suspected diffuse forms showed that 3 and 8 were paternally inherited respectively. One suspected diffuse form had a maternally inherited mutation and corresponded to a dominant form. Except for this case, all patients bearing one mutation in a K_{ATP} channel gene had parents with no clinical history of hypoglycaemia. In addition diabetes was not reported in parents and second-degree relatives of the probands, as originally described in dominant K_{ATP} CHI.(21) These two arguments, the recurrence of CHI in two

siblings with asymptomatic parents and the fact that dominant inactivating *ABCC8* or *KCNJ11* mutation are usually associated with diazoxide-responsive forms of CHI (21, 38) are not in favour of a dominant inheritance that would explain the cases with a single K_{ATP} heterozygous mutation. Nevertheless, these observation could be related to a non penetrance as reported for dominant forms of CHI and transient neonatal diabetes associated with K_{ATP} channel mutations.(38-39) Thirdly, despite an exhaustive molecular analysis including the search for point mutations and for genomic rearrangements, we cannot exclude that rare variants located in intronic and regulatory regions which are not usually screened may be involved. However, the analysis of affected siblings showed that they did not share the same maternal haplotype and excluded a recessive inheritance. Lastly, another hypothesis may be the occurrence of a post-zygotic mutation. The recurrence of CHI in the aforementioned affected siblings could only be explained by the occurrence of independent post-zygotic events. Unfortunately, pancreatic material was not available for these patients. However, the exclusion of somatic point mutations in the pancreatic tissue of four patients was not in favour of the involvement of coding post-zygotic events as a general molecular mechanism. Nevertheless, the sensitivity of sequence analysis would not allow identifying mutations restricted to the endocrine tissue or with a low level of mosaicism.

The predominance of paternally inherited mutations in suspected diffuse forms remains unexplained and needs to be confirmed on larger series. Interestingly, Fernandez-Marmiesse *et al* reported five diazoxide-unresponsive CHI patients who underwent surgery and whose histology did not show a focal lesion. All five inherited the mutation from their father.(34) Epigenetic anomalies could be involved since *ABCC8* and *KCNJ11*, which are not known to be imprinted genes, are located in the vicinity of the 11p15.5 imprinted region. DNA methylation abnormalities of the 11p15.5 locus are observed in Beckwith-Wiedemann syndrome, a congenital overgrowth syndrome that can be associated with

hyperinsulinism.(40) Preliminary experiments performed in six patients did not show evidence of abnormal methylation at the *H19*, *IFG2* and *KCNQ10T1* loci in DNA extracted from leucocytes (data not shown). Further experiments should be done on pancreatic sections of these diffuse forms to exclude somatic epigenetic defects.

In the third and last group comprising patients with diffuse (n=3), suspected diffuse (n=9) or atypical (n=8) forms, neither a point mutation nor a large rearrangement in *ABCC8*/*KCNJ11* was found. This third group of patients seems to have a less severe phenotype. Only 25% were large for gestational age and 40% were refractory to octreotide therapy. We screened the *GCK* gene since three *de novo* activating *GCK* mutations have been reported in patients unresponsive to diazoxide.(41, 42) We found one novel *de novo GCK* mutation which is predicted to be pathogenic and which altered an amino acid previously reported to be the target of a mutation in a patient with CHI.(43) This confirms the low prevalence of *GCK* activating mutations in severe forms of CHI. Even though the proportion of CHI patients with unexplained molecular aetiology was significantly decreased compared to previous studies, unknown genes remain to be identified in CHI refractory to diazoxide.

Finally, a mosaic paternal uniparental disomy (UPD) has been reported in an "atypical diffuse" form of CHI with a paternally inherited *ABCC8* mutation.(17) Contrary to focal forms characterized by a paternal UPD of the 11p15.1-15.5 imprinted region, this atypical form was related to a paternal UPD observed both in DNA from leucocytes and pancreatic tissue but restricted to the 11p15.1 region. We excluded a similar molecular mechanism in our cases of atypical CHI.

In conclusion, 82% of patients with CHI unresponsive to diazoxide were related to *ABCC8* or *KCNJ11* mutations in our series. The molecular analysis alone provides an informative genetic diagnosis for the clinical management of CHI patients with recessively inherited

pathogenic mutations. Genetic counselling and prenatal diagnosis may be offered to families with such previous CHI cases. In contrast, in patients with a single K_{ATP} channel mutation, the molecular analysis should be systematically confronted with the parental segregation analysis and the PET imaging diagnosis. If the mutation is *de novo* or paternally-inherited and the PET diagnosis in favour of a focal form, the surgery will be offered for the resection of the focal lesion. But, when the PET imaging suspects a diffuse form, the molecular diagnosis has a limited added value for the clinical management of CHI patients.

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TABLES

Table 1: Relationships between the *ABCC8/KCNJ11* genotype and the radiopathological diagnosis

Table 2: Characteristics of *ABCC8* and *KCNJ11* mutations

Table 1 Correlations between the *ABCC8/KCNJ11* genotype and the radiopathological diagnosis

	Histopathological diagnosis			Radiological diagnosis[#]		
	Focal	**Diffuse**	**Atypical**	**Focal (PET/PVS)**	**Diffuse (PET/PVS)**	**Total**
n	35	27	8	2	37	109
2 mutations, n (%) *ABCC8/KCNJ11*, n	0	14 (52%) 14 / 0	0	0	16 (43%) (12/4) 13 / 3	30 (28%)
1 mutation, n (%) *ABCC8/KCNJ11*, n	35 32 / 3	10 (37%) 8 / 2	0	2 (2/0) 0 / 2	12 (32%) (12/0) 12 / 0	59 (54%)
Paternal inheritance, n	*22*	*3*		*2*	*8*	35
Maternal inheritance, n	*0*	*0*		*0*	*1*	1
De novo, n	*2*	*2*		*0*	*1*	5
Absent in the mother[$], n	*1*	*1*		*0*	*1*	3
Unknown inheritance, n	*10*	*4*		*0*	*1*	15
No *ABCC8/KCNJ11* mutation, n (%)	0	3 (11%) *	8	0	9 (24%) (7/2)	20 (18%)

[#]non operated patients; n, number of patients, [$] paternal sample unavailable, * 1 patient had a *GCK* mutation

Table 2: Characteristics of *ABCC8* and *KCNJ11* mutations

Gene	Location	Nucleotide sequence change	Protein effect	Occurrence	Histopathological / Radiological diagnosis[‡]	Genetic Status[$]	References
ABCC8	Exon 1	c.62T>A	p.Val21Asp	1	D_{PVS}	hmz	Sandal et al, 2009
ABCC8	Exon 2	c.221G>A	p.Arg74Gln	1	D_H	c-htz	Flanagan et al, 2008
ABCC8	Exon 2	c.259_268del	p.Cys87fs	1	F_H		This report
ABCC8	Exon 3	c.403C>G	p.Leu135Val	1	D_H	c-htz	This report
ABCC8	Exon 4	c.428G>A	p.Trp143X	3	F_H, D_H, D_{PET}	c-htz (x2)	This report
ABCC8	Exon 4	c.496C>T	p.Gln166X	1	D_{PET}	c-htz	This report
ABCC8	Exon 4	c.536A>G	p.Tyr179Cys	2	F_H, D_{PET}	hmz	Damaj et al, 2008
ABCC8	Intron 4	c.580-1G>C	p.?	1	D_H	c-htz	This report
ABCC8	Exon 5	c.655C>T	p.Gln219X	2	D_{PET}, D_{PVS}	c-htz;htz^P	Flanagan et al, 2008
ABCC8	Exon 5	c.683G>A	p.Gly228Asp	1	F_H		Flanagan et al, 2008
ABCC8	Exon 5	c.727_756del30	p.Lys243_Lys252del	1	D_H	hmz	This report
ABCC8	Exon 5	c.742C>T	p.Arg248X	1	D_{PET}	hmz	Flanagan et al, 2008
ABCC8	Exon 6	c.950delC	p.Pro317fs	1	D_{PET}	c-htz	This report
ABCC8	Exon 7	c.1176G>A	p.?	1	D_{PET}	htz^P	Flanagan et al, 2008
ABCC8	Exon 8	c.1177-?_1332+?del	p.Thr393_Gln444del52	1	D_H	c-htz	This report
ABCC8	Exon 8	c.1331A>G	p.Gln444Arg	1	F_H		Damaj et al, 2008
ABCC8	Exon 10	c.1508T>C	p.Leu503Pro	1	F_H		Flanagan et al, 2008
ABCC8	Exon 10	c.1531C>A	p.Leu511Met	2	D_H	htz, htz^{novo}	This report
ABCC8	Intron 10	c.1630+1G>T	p.?	3	F_H (x2), D_{PET}	htz^P	Flanagan et al, 2008
ABCC8	Exon 12	c.1732_1746dup15	p.Ala578_Leu582dup5	1	D_{PET}	htz^P	Flanagan et al, 2008
ABCC8	Exon 12	c.1738C>T	p.Leu580Phe	1	D_{PET}	hmz	This report
ABCC8	Exon 12	c.1792C>T	p.Arg598X	2	F_H, D_H	c-htz	Flanagan et al, 2008
ABCC8	Intron 13	c.1923+5G>T	p.?	1	F_H		This report
ABCC8	Exon 14	c.2035_2036insCTGT	p.Val679fs	1	D_H	hmz	This report

ABCC8	Exon 15	c.2051G>A	p.Gly684Glu	1	F_H		This report
ABCC8	Exon 15	c.2064G>A	p.Trp688X	1	F_H		Giurgea et al, 2004
ABCC8	Intron 15	c.2116+2T>C	p.?	1	D_H	c-htz	This report
ABCC8	Exon 16	c.2124_2127delGACT	p.Thr709X	1	F_H		This report
ABCC8	Exon 16	c.2147G>A	p.Gly716Asp	1	D_{PET}	htz^m	This report
ABCC8	Exon 16	c.2153delG	p.Gly718fs	1	D_H	htz^P	This report
ABCC8	Exon 20	c.2425C>T	p.Gln809X	1	D_H	c-htz	Damaj et al, 2008
ABCC8	Exon 20	c.2473G>A	p.Glu825Lys	1	D_{PET}	htz^P	This report
ABCC8	Exon 22	c.2560-?_2697+?del	p.Asp854_Trp899del46	1	D_H	c-htz	This report
ABCC8	Exon 22	c.2581G>C	p.Asp861His	1	D_{PVS}	c-htz	This report
ABCC8	Exon 22	c.2669A>C	p.Lys890Thr	1	D_H	htz^P	Flanagan et al, 2008
ABCC8	Exon 22	c.2672T>C	p.Leu891Pro	1	D_H	htz^{novo}	This report
ABCC8	Exon 23	c.2702T>C	p.Ile901Thr	2	D_H, D_{PET}	c-htz	This report
ABCC8	Exon 23	c.2784G>A	p.Trp928X	1	F_H		This report
ABCC8	Exon 23	c.2803C>T	p.Gln935X	1	D_{PET}	c-htz	This report
ABCC8	Exon 24	c.2860C>T	p.Gln954X	1	F_H		Flanagan et al, 2008
ABCC8	Intron 24	c.2924-9G>A	p.?	1	D_{PET}	htz^P	This report
ABCC8	Intron 24	c.2924-2A>G	p.?	2	D_{PET}	hmz	This report
ABCC8	Exon 25	c.2994G>A	p.Trp998X	1	D_H	c-htz	This report
ABCC8	Exon 25	c.3111G>A	p.Trp1037X	1	F_H		This report
ABCC8	Exon 25	c.3133_3152del20	p.Thr1045fs	2	F_H, D_{PVS}	c-htz	Flanagan et al, 2008*
ABCC8	Exon 27	c.3391A>C	p.Thr1131Pro	1	D_{PVS}	c-htz	Flanagan et al, 2008
ABCC8	Exon 29	c.3577delG	p.Asp1193fs	1	D_{PET}	c-htz	Flanagan et al, 2008
ABCC8	Exon 29	c.3644G>A	p.Arg1215Gln	2	F_H, D_H	hmz	Flanagan et al, 2008
ABCC8	Exon 30	c.3751C>T	p.Arg1251X	1	D_H	c-htz	Flanagan et al, 2008
ABCC8	Intron 32	c.3991+2_3991+15del14	p.?	1	F_H		Flanagan et al, 2008
ABCC8	Intron 32	c.3992-3C>G	p.?	1	F_H		Flanagan et al, 2008
ABCC8	Intron 32	c.3992-9G>A	p.?	1	F_H		Flanagan et al, 2008

ABCC8	Exon 33	c.4040_4045delTCCAGA	p.Ile1347_Gln1348del	1	F_H		Damaj et al, 2008
ABCC8	Exon 33	c.4092C>G	p.His1364Gln	1	F_H		This report
ABCC8	Exon 34	c.4126G>A	p.Gly1376Arg	1	F_H		This report
ABCC8	Exon 34	c.4150G>A	p.Gly1384Arg	1	D_H	c-htz	This report
ABCC8	Exon 34	c.4154_4155delAG	p.Lys1385fs	1	F_H		Flanagan et al, 2008
ABCC8	Exon 34	c.4160C>T	p.Ser1387Phe	1	D_{PET}	htz	Flanagan et al, 2008
ABCC8	Exon 34	c.4166C>A	p.Ser1389Tyr	1	D_{PET}	htz	This report
ABCC8	Exon 34	c.4169T>C	p.Leu1390Pro	1	D_H	htz	Flanagan et al, 2008
ABCC8	Exon 34	c.4177T>A	p.Phe1393Ile	1	F_H		This report
ABCC8	Exon 35	c.4213_4215delATT	p.Ile1405del	2	D_H, D_{PET}	c-htz	This report
ABCC8	Exon 35	c.4228A>T	p.Ile1410Phe	1	D_H	c-htz	This report
ABCC8	Exon 35	c.4241C>T	p.Pro1414Leu	1	F_H		Flanagan et al, 2008
ABCC8	Exon 35	c.4255C>T	p.Arg1419Cys	1	D_{PVS}	c-htz	This report
ABCC8	Exon 35	c.4261C>T	p.Arg1421Cys	1	D_{PET}	c-htz	Flanagan et al, 2008
ABCC8	Exon 35	c.4300G>A	p.Gly1434Ser	1	D_H	c-htz	This report
ABCC8	Exon 35	c.4309C>G	p.Arg1437Gly	1	D_H	c-htz	This report
ABCC8	Exon 36	c.4325delC	p.Pro1442fs	1	F_H		This report
ABCC8	Exon 36	c.4345_4347dupAGC	p.Ser1449dup	1	D_H	c-htz	This report
ABCC8	Exon 36	c.4352T>G	p.Leu1451Arg	1	D_{PVS}	c-htz	Flanagan et al, 2008
ABCC8	Exon 36	c.4372G>A	p.Ala1458Thr	1	F_H		Flanagan et al, 2008
ABCC8	Exon 36	c.4372G>C	p.Ala1458Pro	1	D_H	hmz	This report
ABCC8	Exon 36	c.4373C>T	p.Ala1458Val	1	D_{PET}	htz^P	This report
ABCC8	Exon 36	c.4390delG	p.Val1464X	1	D_{PET}	htz^P	This report
ABCC8	Exon 36	c.4414G>A	p.Asp1472Asn	1	F_H		Flanagan et al, 2008
ABCC8	Exon 36	c.4414G>C	p.Asp1472His	1	D_H	c-htz	Henwood et al, 2005
ABCC8	Intron 36	c.4415-13G>A	p.?	1	F_H		Flanagan et al, 2008
ABCC8	Exon 37	c.4442A>T	p.Asn1481Ile	1	D_{PET}	htz^{novo}	This report
ABCC8	Exon 37	c.4480C>T	p.Arg1494Trp	1	F_H		Flanagan et al, 2008

ABCC8	Exon 37	c.4481G>A	p.Arg1494Gln	1	D_H	htz	Flanagan et al, 2008
ABCC8	Exon 37	c.4518C>G	p.Asp1506Glu	1	D_H	htz	This report
ABCC8	Intron 38	c.4612-2A>T	p.?	1	D_H	c-htz	Flanagan et al, 2008
KCNJ11	Exon 1	c.101G>A	p.Arg34His	1	D_{PET}	hmz	Flanagan et al, 2008
KCNJ11	Exon 1	c.383A>G	p.Gln128Arg	1	F_H		This report
KCNJ11	Exon 1	c.406C>T	p.Arg136Cys	1	F_{PET}		This report
KCNJ11	Exon 1	c.492_493insGGTT	p.Cys166fs	1	D_{PET}	c-htz	This report
KCNJ11	Exon 1	c.637G>A	p.Ala213Thr	1	F_H		This report
KCNJ11	Exon 1	c.667A>C	p.Thr223Pro	1	D_H	htz	This report
KCNJ11	Exon 1	c.777T>G	p.His259Gln	1	F_H		This report
KCNJ11	Exon 1	c.881C>T	p.Thr294Met	2	D_H, D_{PET}	c-htz;htz^P	Shimomura et al, 2009
KCNJ11	Exon 1	c.901C>T	p.Arg301Cys	1	F_{PET}		Lin et al, 2008
KCNJ11	Exon 1	c.902G>A	p.Arg301His	1	D_{PET}	c-htz	Flanagan et al, 2008
KCNJ11	Exon 1	c.998T>C	p.Phe333Ser	1	D_{PET}	c-htz	This report

* Reported as c.3130_3149del20; $ Genetic status for diffuse forms; hmz: homozygous; c-htz: compound heterozygous; htz: heterozygous without information on the inheritance; htz^p for paternally inherited, htz^m for maternally inherited and htz^{novo} for *de novo* mutations; ‡ D and F stand for diffuse and focal forms, respectively. When the diagnosis was based on histological data, H was added in lowercase. When only radiological diagnosis was available, the corresponding technique (PET, PVS) was indicated in lowercase.